

Figure 1

Schematic Diagram of HPLC-EC System

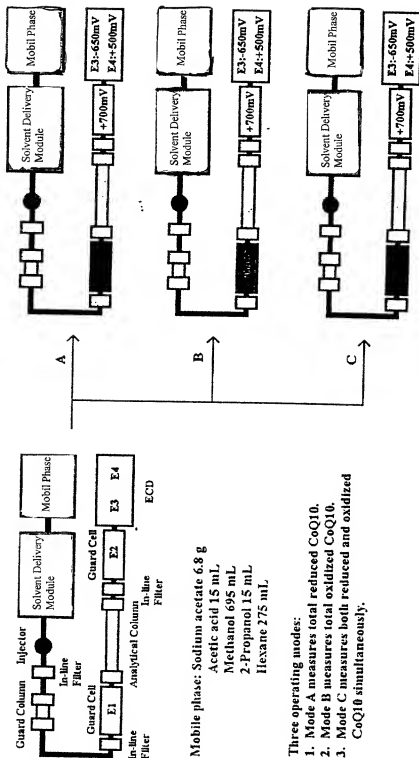


Figure 2.

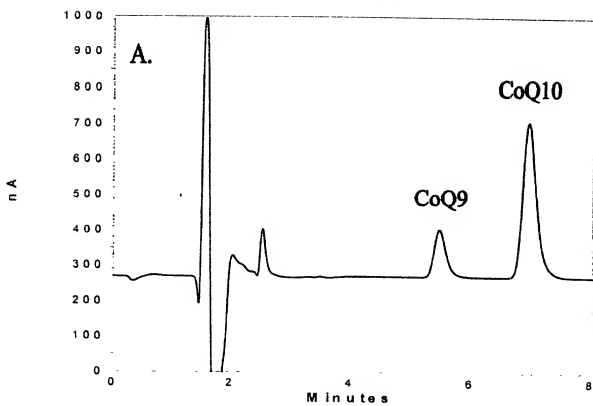
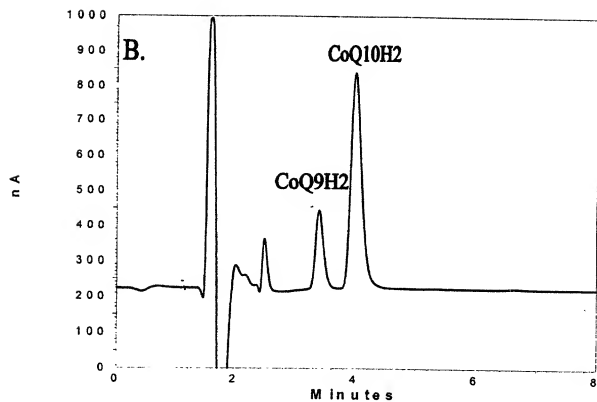


Figure 3.

Calibration Curves for Reduced and
Oxidized CoQ10

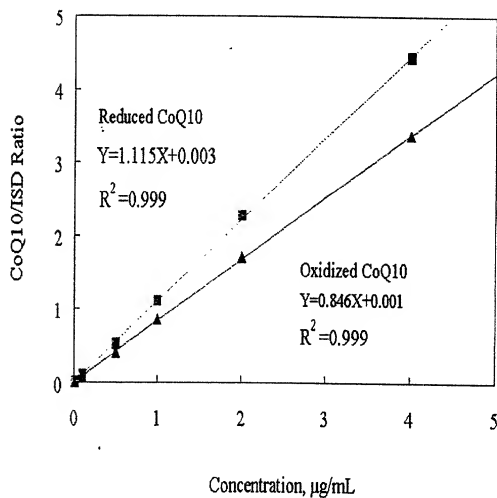


Figure 4.

Effect of Heparin and EDTA on CoQ₁₀H₂ Stability

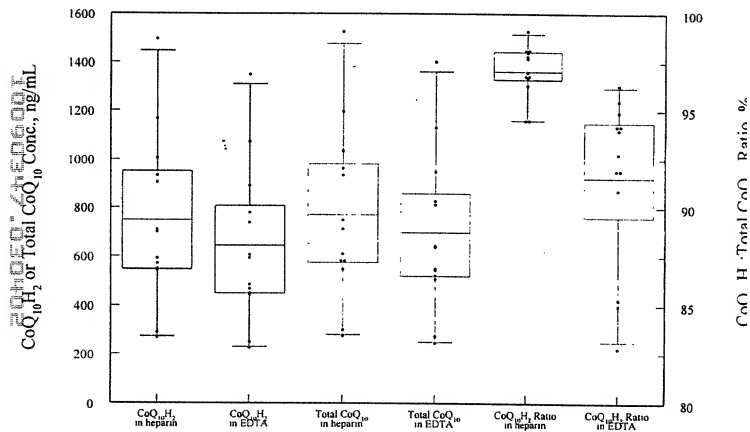


Figure 5.

Comparison Between EDTA and Heparin (Blood Specimen Stored at +4 °C)

△ EDTA ● Heparin □ Heparin Gel

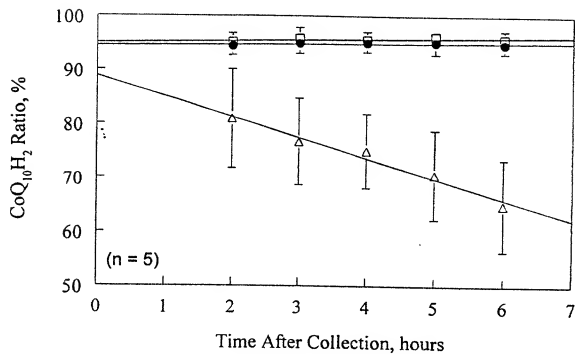


Figure 6.

Stability of $\text{CoQ}_{10}\text{H}_2$ in Heparin Vacutainer® (Blood Specimens Stored at 4°C)

△ Sample 1 □ Sample 2 ● Sample 3 + Sample 4

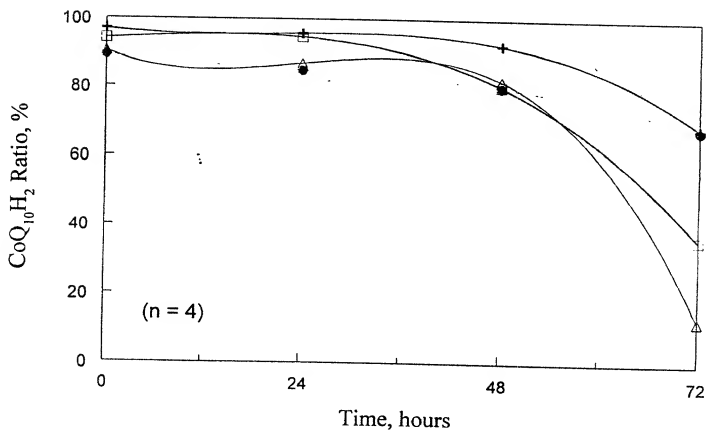


Figure 7.

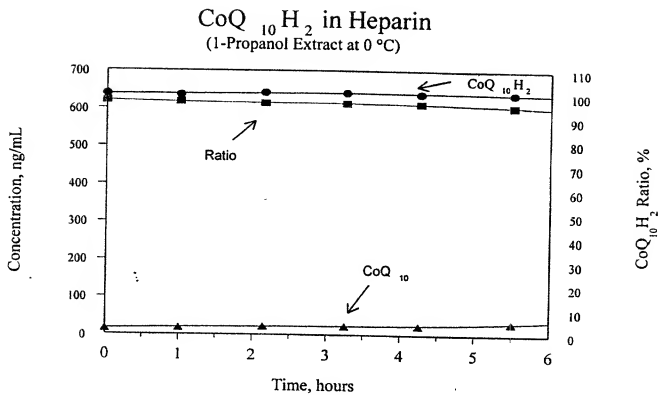


Figure 8.

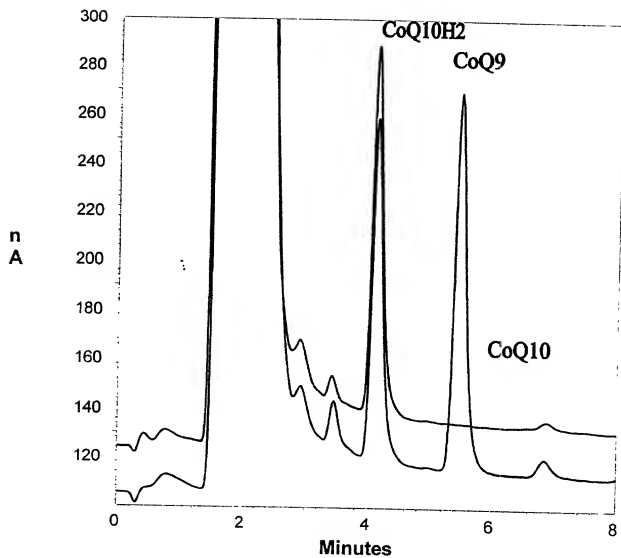


Figure 9.

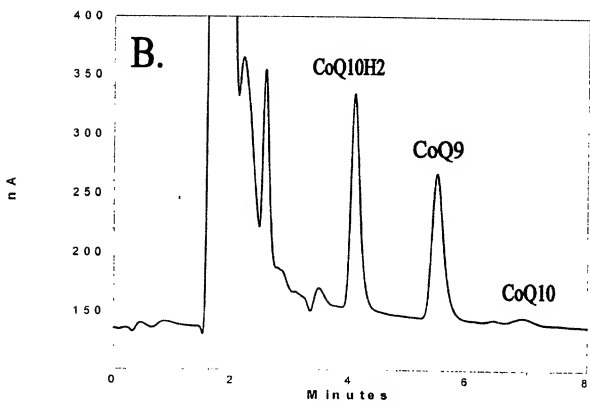
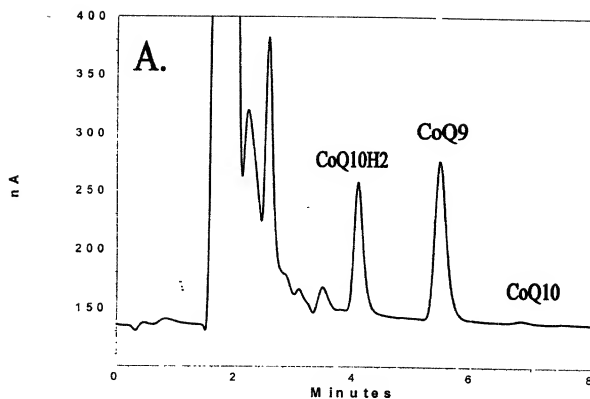


Figure 10.

Table 1. Comparison between current and previous studies on $\text{CoQ}_{10}\text{H}_2$ analysis.

| Ref. # | Blood Sample | Sample Size, μL | Extraction Solvent | Precolumn Reduction | Internal Standard | HPLC Run Time, min | $\text{CoQ}_{10}\text{H}_2$ Ratio, % | Configuration |
|---------|-----------------|----------------------------|--------------------|-----------------------------------|------------------------------------|--------------------|--------------------------------------|-----------------------------|
| 1 | Heparin | 1000 | Ethanol/hexane | NaBH_4 | CoQ_9 | 10 | 51.1 \pm 4.2 | Amperometric detector |
| 2 | Heparin | 200 | Hexane | $\text{Na}_2\text{S}_2\text{O}_4$ | CoQ_9 | 14 | N/A | Amperometric detector |
| 3 | Heparin | 300 | 1-Propanol | NaBH_4 | Diethoxy CoQ_{10} | 11 | N/A | Coupled-column/EC-UV |
| 4 | Heparin | 200 | Hexane | NaBH_4 | CoQ_9 | 16 | 87.0 \pm 1.0 | Amperometric detector |
| 5 | EDTA | 1000 | Hexane/SPE | EC | CoQ_9 | 10 | N/A | SPE, column-switching |
| 6 | Serum | 100 | Hexane | NaBH_4 | CoQ_9 | 15 | 65.8 \pm 4.2 | Amperometric detector |
| 7 | Heparin | 10 | Hexane | $\text{Na}_2\text{S}_2\text{O}_4$ | CoQ_9 | 20 | 93.6 \pm 3.0 | Coulometric detector |
| 8 | EDTA | 300 | 1-Propanol | NaBH_4 | None | 13 | 94.3 \pm 0.7 | Post-column valve switching |
| 9 | Heparin | 50 | Methanol/hexane | NaBH_4 | CoQ_9 | 22 | 91.5 \pm 5.7 | Amperometric detector |
| 10 | Heparin EDTA | 500 | Methanol/hexane | EC | CoQ_9 , CoQ_{10} | 15-20 | 88.6 \pm 1.0 | Amperometric detector |
| 11 | EDTA | 100 | 2-Propanol | NaBH_4 | None | 13 | -93 | Amperometric detector |
| Current | Heparin | 100 | 1-Propanol | EC | CoQ_9 | 8 | 96.6 \pm 2.2 | Coulometric detector |

Table 2. Electrochemical reduction of CoQ₁₀

| Concentration ng/mL | CoQ10H2 ng/mL | Unchanged CoQ10 ng/mL | Conversion Rate % |
|------------------------|------------------|--------------------------|----------------------|
| 10 | 10 | 0 | 100 |
| 100 | 100 | 0 | 100 |
| 1000 | 989 | 11 | 98.9 |
| 1500 | 1485 | 15 | 99.0 |
| 2000 | 1982 | 18 | 99.1 |
| 3000 | 2977 | 23 | 99.2 |
| 4000 | 3974 | 26 | 99.3 |
| (n=2) | | | Mean = 99.4 |

Table 3. Extraction recoveries of CoQ₁₀ and CoQ₉ using different solvents

| Compound | Extraction Solvent | Volume (μL) | Recovery mean, % | n |
|-------------------|--------------------------|----------------|------------------|---|
| | | Plasma:Solvent | | |
| CoQ ₁₀ | 1-Propanol | 100-900 | 100 | 6 |
| CoQ ₉ | 1-Propanol | 100-900 | 100 | 6 |
| CoQ ₁₀ | 2-Propanol/1-Propanol | 100:850:50 | 89 | 2 |
| CoQ ₉ | 2-Propanol/1-Propanol | 100:850:50 | 100 | 2 |
| CoQ ₁₀ | Methanol/1-Propanol | 100:850:50 | 19 | 2 |
| CoQ ₉ | Methanol/1-Propanol | 100:850:50 | 35 | 2 |
| CoQ ₁₀ | Ethanol/1-Propanol | 100:850:50 | 88 | 2 |
| CoQ ₉ | Ethanol/1-Propanol | 100:850:50 | 96 | 2 |
| CoQ ₁₀ | n-Butanol/1-Propanol | 100:850:50 | 85 | 2 |
| CoQ ₉ | n-Butanol/1-Propanol | 100:850:50 | 91 | 2 |
| CoQ ₁₀ | Acetone/1-Propanol | 100:850:50 | 71 | 2 |
| CoQ ₉ | Acetone/1-Propanol | 100:850:50 | 87 | 2 |
| CoQ ₁₀ | Acetonitril/1-Propanol | 100:850:50 | 19 | 2 |
| CoQ ₉ | Acetonitril/1-Propanol | 100:850:50 | 50 | 2 |
| CoQ ₁₀ | Ethanol/Hexane (2:5 v/v) | 100-900 | 98 | 6 |
| CoQ ₉ | Ethanol/Hexane (2:5 v/v) | 100-900 | 100 | 6 |

Table 4. Precision Data for CoQ₁₀ analysis.

| Intended Conc. ng/mL | Measured Conc. ng/mL | R.S.D. % | Recovery % |
|----------------------------------|-------------------------|-------------|---------------|
| Within-Day Precision (n=6) | | | |
| 75 | 74±3 | 3.6 | 99.3 |
| 450 | 431±11 | 2.7 | 95.8 |
| 1200 | 1171±14 | 1.2 | 97.6 |
| Day-Day Precision (n=54, 9 days) | | | |
| 75 | 76±4 | 4.9 | 100.7 |
| 450 | 437±19 | 4.3 | 97.1 |
| 1200 | 1174±28 | 2.4 | 97.8 |